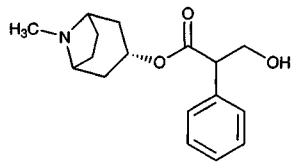
Atropine



Molecular formula: $C_{17}H_{23}NO_3$
Molecular weight: 289.37
CAS Registry No.: 51-55-8, 52-88-0 (atropine methylnitrate)
Merck Index: 907
Lednicer No.: 1 35, 71, 93; 2 71

SAMPLE

Matrix: blood
Sample preparation: 1 mL Plasma + 100 μL 5 ng/mL scopolamine in MeOH, vortex briefly, add 50 μL 1 M ammonium hydroxide, mix, add 5 mL dichloromethane, shake horizontally for 5 min, centrifuge at 2500 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue in 100 μL mobile phase, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 10 × 2 5 μm BDS C18 (Keystone)
Column: 50 × 3 3 μm BDS C18 (Keystone)
Mobile phase: MeCN:MeOH:10 mM ammonium acetate 62.5:37.5:15
Flow rate: 0.5
Injection volume: 20
Detector: MS, Perkin Elmer Sciex API III-Plus triple quadrupole, APCI, nebulizer 400° and 80 psi, auxiliary nitrogen 1.2 L/min, curtain gas 1.2 L/min, interface 55°, collision gas argon, electron multiplier 3000 V, declustering potential 35 V, collision energy 35 eV

CHROMATOGRAM

Retention time: 1.2
Internal standard: scopolamine (0.8)
Limit of quantitation: 20 pg/mL

KEY WORDS

plasma; protect from light

REFERENCE

Xu,A.; Havel,J.; Linderholm,K.; Hulse,J. Development and validation of an LC/MS/MS method for the determination of L-hyoscyamine in human plasma, *J.Pharm.Biomed.Anal.*, **1996**, *14*, 33–42.

SAMPLE

Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.

Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 200.5

CHROMATOGRAM
Retention time: 10.388

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: bulk, plants
Sample preparation: Place 0.5 g powdered crude drug in 25 mL mobile phase, reflux 30 min, cool, centrifuge at 1600 g, decant wash residue twice with 10 mL portions of mobile phase, combine extracts and washings, make up to 50 mL with mobile phase, inject 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 5 μm TSK gel 120A ODS
Mobile phase: MeCN:67 mM pH 2.5 phosphate buffer 35:65 containing 17.5 mM sodium dodecylsulfate
Column temperature: 35
Flow rate: 1.5
Injection volume: 10
Detector: UV 210

CHROMATOGRAM
Retention time: 15

OTHER SUBSTANCES
Simultaneous: scopolamine

REFERENCE
Oshima,T.; Sagara,K.; Tong,Y.Y.; Zhang,G.; Chen,Y.H. Application of ion-pair high performance liquid chromatography for analysis of hyoscyamine and scopolamine in solanaceous crude drugs, *Chem.Pharm.Bull.(Tokyo)*, **1989**, *37*, 2456–2458.

SAMPLE
Matrix: formulations
Sample preparation: Oral solutions. Dilute an amount oral solution equivalent to 25 μg atropine sulfate to 10 mL with EtOH. Tablets. Weight out finely powdered tablets equivalent to 25 μg atropine sulfate, add 10 mL MeCN:water 50:50, sonicate with frequent swirling for 15 min. Filter (0.45 μm membrane filter), discard the first portion. Inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb CN
Mobile phase: A:B 34:66 (Prepare A as follows. Dissolve 192 mg 1-pentanesulfonic acid sodium salt monohydrate in 200 mL water. Add 800 mL water and 1 mL orthophosphoric

acid. Prepare B as follows. Dissolve 192 mg 1-pentanesulfonic acid sodium salt monohydrate in 200 mL water. Add 800 mL MeCN and 1 mL orthophosphoric acid.)
Flow rate: 1.7
Injection volume: 50
Detector: UV 220

CHROMATOGRAM
Retention time: 4.8

OTHER SUBSTANCES
Simultaneous: metabolites, diphenoxylate

KEY WORDS
oral solutions; tablets

REFERENCE
Lehr,G.J. Determination of diphenoxylate hydrochloride and atropine sulfate in combination drug formulations by liquid chromatography, *J.AOAC Int.*, **1996**, *79*, 1288–1293.

SAMPLE
Matrix: formulations
Sample preparation: Tablets, capsules. Powder tablets or remove contents of capsules, weigh out amount equivalent to about 600 μg hyoscyamine sulfate-atropine sulfate, add 25 mL 25 mM sulfuric acid, shake for 15 min, centrifuge at 3000 rpm for 5 min. Remove 5 mL of the supernatant and extract it twice with 30 mL portions of dichloromethane, discard the organic phase, add 2 mL buffer to the aqueous phase, extract with four 30 mL portions of dichloromethane, filter extracts through dichloromethane-rinsed glass wool, add 3 mL 2.25 μg/mL theophylline in dichloromethane, distil off the dichloromethane through a Snyder column by using a steam bath, when the volume reaches 10 mL rinse the column with 1-2 mL dichloromethane, continue distillation to 0.5-1 mL, remove the column and rinse the concentrator tube-column junction with 1 mL dichloromethane, evaporate to 1 mL with a stream of air at 40°, add 100 μL 1% concentrated HCl in MeOH, mix, evaporate to dryness with a stream of air at 40°, rinse the sides of the concentrator tube with 500 μL MeOH, evaporate to dryness with a stream of air at 40°, dissolve the residue in 300 μL water, inject a 20 μL aliquot. Elixirs. Add an amount equivalent to about 600 μg hyoscyamine sulfate-atropine sulfate to a 150 mL beaker, warm at 40° with a current of air for 30 min to remove alcohol, cool, make up to 25 mL with water, remove 5 mL of this solution, add 2 mL 100 mM sulfuric acid, extract twice with 30 mL portions of dichloromethane, discard the organic phase, add 2 mL buffer to the aqueous phase, extract with four 30 mL portions of dichloromethane, filter extracts through dichloromethane-rinsed glass wool, add 3 mL 2.25 μg/mL theophylline in dichloromethane, distil off the dichloromethane through a Snyder column by using a steam bath, when the volume reaches 10 mL rinse the column with 1-2 mL dichloromethane, continue distillation to 0.5-1 mL, remove the column and rinse the concentrator tube-column junction with 1 mL dichloromethane, evaporate to 1 mL with a stream of air at 40°, add 100 μL 1% concentrated HCl in MeOH, mix, evaporate to dryness with a stream of air at 40°, rinse the sides of the concentrator tube with 500 μL MeOH, evaporate to dryness with a stream of air at 40°, dissolve the residue in 300 μL water, inject a 20 μL aliquot. (Buffer was 5.3 g anhydrous sodium carbonate and 4.2 g sodium bicarbonate in 100 mL water, pH 9.4. Pass dichloromethane through 75 g basic aluminum oxide, Brockmann Activity Grade 1, store over 25 g alumina/4 L.)

HPLC VARIABLES
Column: 250 × 4 5 μm Spherisorb ODS
Mobile phase: MeOH:buffer 250:525 (The 50 mM tetramethylammonium phosphate buffer was prepared from 500 mL water + 23 mL 20% tetramethylammonium hydroxide in MeOH + 10 mL concentrated phosphoric acid, adjust to pH 2.0 with concentrated phosphoric acid, make up to 1 L with water.)

Flow rate: 0.8
Injection volume: 20
Detector: UV 220

CHROMATOGRAM
Retention time: 7.5
Internal standard: theophylline (6.5)

OTHER SUBSTANCES
Simultaneous: phenobarbital, scopolamine, atropine

KEY WORDS
tablets; capsules; elixirs

REFERENCE
Pennington,L.J.; Schmidt,W.F. Belladonna alkaloids and phenobarbital combination pharmaceuticals analysis I: High-performance liquid chromatographic determinations of hyoscyamine-atropine and scopolamine, *J.Pharm.Sci.*, **1982**, *71*, 951–953.

SAMPLE
Matrix: formulations
Sample preparation: Grind tablets to a fine powder, weigh out amount containing 25 μg atropine, extract with 40 mL chloroform, filter, wash filter with chloroform, make up filtrate to 50 mL with chloroform, mix. Remove a 1 mL aliquot and evaporate it to dryness under reduced pressure at 40°, reconstitute with 500 μL 4 mg/mL quinuclidine in acetone, add 500 μL 2 mg/mL 1-anthroylnitrile in acetone, heat at 30° for 10 min, add 1 mL 2% phosphoric acid, cool to room temperature, make up to 10 mL with acetone, inject a 10 μL aliquot. (1-Anthroylnitrile is available from Wako Chemicals, Richmond VA. Synthesis is as follows. Dissolve 50 g benzanthrone in 500 mL concentrated sulfuric acid with gentle warming, pour this solution cautiously into 4 L hot water with vigorous stirring. Boil the suspension and slowly add 200 g chromium(VI) oxide (Caution! Chromium oxide is a carcinogen and highly corrosive!), after 6 h cool the mixture, filter, wash the precipitate with hot water. Dissolve the precipitate in dilute ammonia and precipitate with acid, crystallize from boiling concentrated nitric acid to give anthraquinone-1-carboxylic acid (Ber. 1924, 57, 1775). Warm, on a water bath, anthraquinone-1-carboxylic acid in dilute ammonia with twice the amount of zinc dust, when the reaction has ceased (30 min ?) filter the reaction the reaction mixture, add HCl to the filtrate to obtain anthracene-1-carboxylic acid as yellow needles, recrystallize from EtOH (mp 245°) (Ber 1897, 30, 1118). Stir 1 g anthracene-1-carboxylic acid in 15 mL anhydrous dichloromethane, add 2 mL oxalyl chloride, reflux for 1 h, evaporate to give 1-anthroyl chloride as an oily residue. Dissolve 1-anthroyl chloride in 15 mL dichloromethane, add 3 mL trimethylsilyl cyanide, add 1 mg zinc iodide, stir at room temperature for 2 h, evaporate to dryness, recrystallize from hexane/dichloromethane to give 1-anthroylnitrile as orange-yellow needles (mp 164-5°) (Anal.Chim.Acta 1983, 147, 397).)

HPLC VARIABLES
Column: 150 × 4.6 5 μm Cosmocil 5C-18 (Nacalai Tesque, Tokyo)
Mobile phase: MeCN:buffer 60:40 (Buffer was 20 mM sodium dodecyl sulfate adjusted to pH 3.5 with phosphoric acid.)
Column temperature: 40
Flow rate: 1
Injection volume: 10
Detector: F ex 255 em 474

CHROMATOGRAM
Retention time: 12
Limit of detection: 10 ng/mL
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: albumin, amomum seed, caffeine, chlorpheniramine, cinnamon bark, cloves, fennel, geranium herb, glycyrrhiza, lysozyme, swertia herb, thiamine, riboflavin

KEY WORDS
derivatization; tablets

REFERENCE
Takahashi,M.; Nagashima,M.; Shigeoka,S.; Nishijima,M.; Kamata,K. Determination of atropine in pharmaceutical preparations by liquid chromatography with fluorescence detection, *J.Chromatogr.A*, **1997**, *775*, 137–141.

SAMPLE
Matrix: plants
Sample preparation: Dissolve alkaloids in 1 mL MeOH, add 40 ng homatropine, inject aliquot.

HPLC VARIABLES
Column: 150 × 4.1 5 μm Hamilton PRP-1
Mobile phase: MeCN:100 mM pH 10.4 ammonium acetate
Flow rate: 1
Injection volume: 20
Detector: MS thermospray, VG Trio-2, ion source 150°, vaporizer tip 170°, repeller electrode 150 V, m/z 290

CHROMATOGRAM
Internal standard: homatropine (m/z 276)
Limit of detection: 2.5 ng/mL

OTHER SUBSTANCES
Simultaneous: scopolamine

KEY WORDS
total run time 6 min

REFERENCE
Auriola,S.; Martinsen,A.; Oksman-Caldentey,K.M.; Naaranlahti,T. Analysis of tropane alkaloids with thermospray high-performance liquid chromatography-mass spectrometry, *J.Chromatogr.*, **1991**, *562*, 737–744.

SAMPLE
Matrix: plants
Sample preparation: Extract 0.1 g dry plant material with 10 mL MeOH for 10 min under reflux, filter, inject aliquot.

HPLC VARIABLES
Guard column: 40 × 4 10 μm Hypersil ODS
Column: 250 × 4 10 μm Hypersil ODS
Mobile phase: MeOH:water 45:55 containing 0.1% phosphoric acid adjusted to pH 7 with triethylamine
Flow rate: 1
Detector: UV 229

CHROMATOGRAM
Retention time: 20.7

OTHER SUBSTANCES
Simultaneous: scopolamine

REFERENCE
Hagemann,K.; Piek,K.; Stöckigt,J.; Weiler,E.W. Monoclonal antibody-based enzyme immunoassay for the quantitative determination of the tropane alkaloid, scopolamine, *Planta Med.*, **1992**, *58*, 68–72.

SAMPLE
Matrix: plants
Sample preparation: 100 mg Freeze-dried powdered plant leaves + 10 mL mobile phase, heat at 40° for 15 min, filter, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 150 × 4 4 μm Novapack C18
Mobile phase: MeCN:water 12.5:87.5 with 0.3% phosphoric acid adjusted to pH 2.2 with triethylamine
Flow rate: 0.8
Injection volume: 20
Detector: UV 204

CHROMATOGRAM
Retention time: 11.7
Limit of detection: 50000 ng/g

OTHER SUBSTANCES
Simultaneous: tropic acid, scopolamine

REFERENCE
Fliniaux,M.-A.; Manceau,F.; Jacquin-Dubreuil,A. Simultaneous analysis of l-hyoscyamine, l-scopolamine and dl-tropic acid in plant material by reversed phase high-performance liquid chromatography, *J.Chromatogr.*, **1993**, *644*, 193–197.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

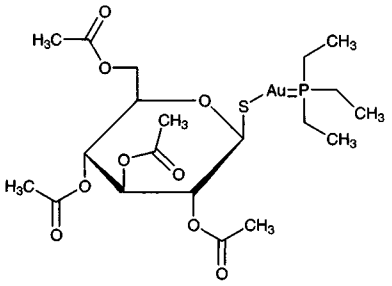
HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 20:1.5:0.5:78
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 4.76

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

Auranofin

Molecular formula: $C_{20}H_{34}AuO_9PS$
Molecular weight: 678.49
CAS Registry No.: 34031-32-8
Merck Index: 911



SAMPLE
Matrix: solutions
Sample preparation: Filter (0.45 or 0.22 μm), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.5 5 μm octadecylsilane (Jones Chromatography)
Mobile phase: MeOH:0.25% ammonium dihydrogen phosphate 65:35
Flow rate: 0.8
Injection volume: 20-50
Detector: UV 214

CHROMATOGRAM
Retention time: 7.5
Limit of detection: 0.2 ppm

KEY WORDS
Krebs-Ringer bicarbonate buffer

REFERENCE
Tepperman,K.; Finer,R.; Donovan,S.; Elder,R.C.; Doi,J.; Ratliff,D.; Ng,K. Intestinal uptake and metabolism of auranofin, a new oral gold-based antiarthritis drug, *Science*, **1984**, *225*, 430–432.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Spherisorb ODS-2
Mobile phase: MeOH:water 50:50 containing 10 mM tetrabutylammonium chloride and 25 mM ammonium formate, pH 6.3
Column temperature: 30
Flow rate: 1
Detector: UV 227 or MS, Sciex Elan 250 ICP-MS, monitor gold 197, RF power 1.4 kW, nebulizer Ar gas flow rate 1 L/min, nebulizer spray chamber 17

CHROMATOGRAM
Retention time: 18
Limit of detection: 0.3 ng

OTHER SUBSTANCES
Also analyzed: myochrysine

REFERENCE
Zhao,Z.; Jones,W.B.; Tepperman,K.; Dorsey,J.G.; Elder,R.C. Determination of gold-based antiarthritis drugs and their metabolites in urine by reversed-phase ion-pair chromatography with ICP-MS detection, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 279–287.

SAMPLE

Matrix: urine
Sample preparation: Filter (0.45 μm), inject a 200 μL aliquot.

HPLC VARIABLES

Guard column: 50 × 4.6 5 μm Spherisorb ODS2
Column: 150 × 4.6 5 μm B & J OD5 octadecyl
Mobile phase: MeOH:water 50:50 containing 10 mM tetrabutylammonium chloride and 25 mM ammonium formate, pH 6
Column temperature: 30
Flow rate: 1
Injection volume: 200
Detector: MS, Sciex Elan 250 ICP-MS, monitor gold 197, RF power 1.4 kW, nebulizer Ar gas flow rate 1 L/min, nebulizer spray chamber 17

CHROMATOGRAM

Retention time: 18
Limit of detection: 500 ng/mL

REFERENCE

Zhao,Z.; Jones,W.B.; Tepperman,K.; Dorsey,J.G.; Elder,R.C. Determination of gold-based antiarthritis drugs and their metabolites in urine by reversed-phase ion-pair chromatography with ICP-MS detection, *J.Pharm.Biomed.Anal.*, **1992**, *10*, 279–287.

SAMPLE

Matrix: urine
Sample preparation: Rabbit urine. Centrifuge at 1000 g at 3° for 1 min, inject an aliquot. Human urine. Inject directly.

HPLC VARIABLES

Column: 150 × 4.6 YMC AM-302 octadecylsilyl (YMC)
Mobile phase: MeOH:water 65:35
Flow rate: 1
Injection volume: 100
Detector: UV 412 with post-column reaction detection. Reagent was 50 μM 5,5'-dithiobis(2-nitrobenzoic acid), 300 mM KI, and 50 mM pH 7.4 phosphate buffer delivered at 0.5 mL/min, mixed with column effluent, passed though a 0.5 mm × 5 m PTFE reaction coil at 60°.

CHROMATOGRAM

Retention time: 6

KEY WORDS

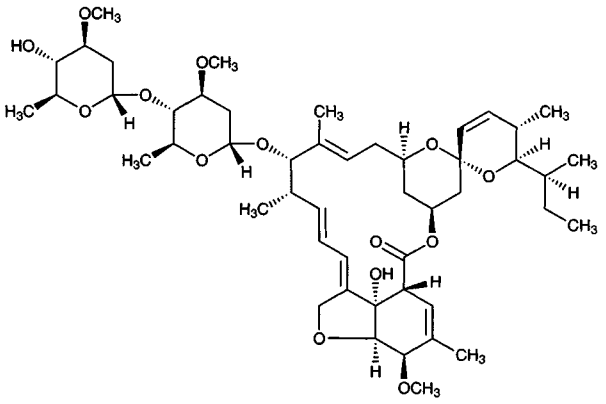
human; rabbit; post-column reaction

REFERENCE

Kizu,R.; Kaneda,M.; Yamauchi,Y.; Miyazaki,M. Determination of auranofin, a chrysotherapy agent, in urine by HPLC with a postcolumn reaction and visible detection, *Chem.Pharm.Bull.*, **1993**, *41*, 1261–1265.

Avermectin

Molecular formula: $C_{48}H_{74}O_{14}$
Molecular weight: 875.11
Merck Index: 919



SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

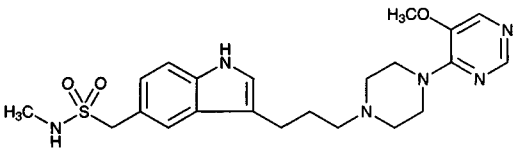
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone, butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, na-

lorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, secobarbital, strychnine, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Avitriptan



Molecular formula: $C_{22}H_{30}N_6O_3S$
Molecular weight: 458.59
CAS Registry No.: 151140-96-4, 171171-42-9 (fumarate)

SAMPLE

Matrix: blood
Sample preparation: Add 50μL 1 M pH 5 ammonium acetate and IS to 500 μL plasma, mix. Add to a conditioned carboxylic acid BondElut SPE cartridge. Wash with pH 5.0 ammonium acetate and dichloromethane. Elute with 2 mL 1% triethylamine in MeOH. Evaporate, reconstitute the residue with 200μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 DeltaBond CN (Keystone Scientific, Bellafonte, PA)
Mobile phase: MeCN:MeOH:water 5:5:90 containing 10 mM pH 3 ammonium phosphate dibasic and 10 mM pH 3 tetramethylammonium hydroxide
Flow rate: 1
Injection volume: 100
Detector: UV 287

CHROMATOGRAM

Retention time: 6.5
Internal standard: BMY-46317 (9)
Limit of quantitation: 10 ng/mL

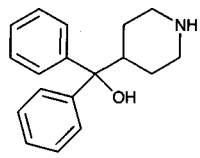
KEY WORDS

pharmacokinetics; radiolabeled; SPE; rat; plasma

REFERENCE

Marathe,P.H.; Greene,D.S.; Barbhaiya,R.H. Disposition of [14C]avitriptan in rats and humans, *Drug Metab.Dispos.*, **1997**, *25*, 881–888.

Azacyclonol



Molecular formula: $C_{18}H_{21}NO$
Molecular weight: 267.37
CAS Registry No.: 115-46-8, 1798-50-1 (HCl)
Merck Index: 925
Lednicer No.: 1 47

SAMPLE

Matrix: solutions
Sample preparation: Prepare a 10 μg/mL solution in MeOH, inject a 20 μL aliquot.

HPLC VARIABLES

Column: 125 × 4.9 Spherisorb S5W silica
Mobile phase: MeOH containing 10 mM ammonium perchlorate and 1 mL/L 100 mM NaOH in MeOH, pH 6.7
Flow rate: 2
Injection volume: 20
Detector: E, LeCarbone, V25 glassy carbon electrode, + 1.2 V

CHROMATOGRAM

Retention time: 1.9

OTHER SUBSTANCES

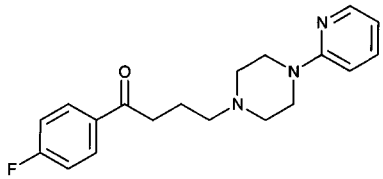
Also analyzed: acebutolol, acepromazine, acetophenazine, N-acetylprocainamide, albuterol, alprenolol, amethocaine, amiodarone, amitriptyline, antazoline, atenolol, bamethan, benactyzine, benperidol, benzethidine, benzocaine, benzoctamine, benzphetamine, benzquinamide, bromhexine, bromodiphenhydramine, bromperidol, brompheniramine, brompromazine, buclizine, bufotenine, bupivacaine, buprenorphine, butacaine, butethamate, chlorcyclizine, chlorpheniramine, chlorphenoxamine, chlorprenaline, chlorpromazine, chlorprothixene, cimetidine, cinchonidine, cinnarizine, clemastine, clomipramine, clonidine, cocaine, cyclazocine, cyclizine, cyclopentamine, cyproheptadine, deserpidine, desipramine, dextromoramide, dextropropoxyphene, dicyclomine, diethylcarbamazine, diethylpropion, diethylthiambutene, dihydroergotamine, dimethindene, dimethothiazine, diphenhydramine, diphenoxylate, dipipanone, diprenorphine, dipyridamole, disopyramide, dothiepin, doxapram, doxepin, doxylamine, droperidol, ephedrine, ergocornine, ergocristine, ergocristinine, ergocryptine, ergometrine, ergosine, ergosinine, ergotamine, ethopropazine, etorphine, etoxeridine, fenethazine, fenfluramine, fenoterol, fentanyl, flavoxate, fluopromazine, flupenthixol, fluphenazine, flurazepam, haloperidol, hydroxyzine, hyoscine, ibogaine, imipramine, indapamine, iprindole, isothipendyl, isoxsuprine, ketanserin, laudanosine, lidocaine, lofepramine, loxapine, maprotiline, mecamylamine, meclophenoxate, meclozine, medazepam, mephentermine, mepivacaine, meptazinol, mepyramine, mesoridazine, metaraminol, methadone, methamphetamine, methapyrilene, methdilazene, methotrimeprazine, methoxamine, methoxyphenamine, methoxypromazine, methylephedrine, methylergonovine, methysergide, metoclopramide, metopimazine, metoprolol, mianserin, morazone, nadolol, nalorphine, naloxone, naphazoline, nicotine, nifedipine, nomifensine, nortriptyline, noscapine, orphenadrine, oxeladin, oxprenolol, oxymetazolin, papaverine, pargyline, pecazine, penbutolol, pentazocine, penthienate, pericyazine, perphenazine, phenadoxone, phenampromide, phenazocine, phenbutrazate, phendimetrazine, phenelzine, phenglutarimide, phenindamine, pheniramine, phenmetrazine, phenomorphan, phenoperidine, phenothiazine, phenoxybenzamine, phentolamine, phenylephrine, phenyltoloxamine, physostigmine, piminodine, pimozide, pindolol, pipamazine, pipazethate, piperacetazine, piperidolate, pipradol, pirenzepine, piritramide, pizotifen, practolol, pramoxine, prazosin, prenylamine, prilocaine, primaquine, proadifen, procainamide, procaine, prochlorperazine, procyclidine, proheptazine, prolintane, promazine, promethazine, pronethalol, properidine, propiomazine, propranolol, prothipendyl,

protriptyline, proxymetacaine, pseudoephedrine, pyrimethamine, quinidine, quinine, ranitidine, rescinnamine, sotalol, tacrine, terazosin, terbutaline, terfenadine, thenyldiamine, theophylline, thiethylperazine, thiopropazate, thioproperazine, thioridazine, thiothixene, thonzylamine, timolol, tocainide, tolpropamine, tolycaine, tranylcypromine, trazodone, trifluoperazine, trifluperidol, trimeperidine, trimeprazine, trimethobenzamide, trimethoprim, trimipramine, tripelennamine, triprolidine, tryptamine, verapamil, xylometazoline

REFERENCE

Jane,I.; McKinnon,A.; Flanagan,R.J. High-performance liquid chromatographic analysis of basic drugs on silica columns using non-aqueous ionic eluents. II. Application of UV, fluorescence and electrochemical oxidation detection, *J.Chromatogr.*, **1985**, *323*, 191–225.

Azaperone



Molecular formula: $C_{19}H_{22}FN_3O$
Molecular weight: 327.40
CAS Registry No.: 1649-18-9
Merck Index: 931
Lednicer No.: 2 300

SAMPLE

Matrix: tissue

Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 5 mL MeOH and 5 mL water. Homogenize kidney with a kitchen grinder. Weigh out a 5 g sample and add 20 mL MeCN with continuous gentle mixing, mix vigorously on a vibromixer at 1500 rpm for 30 s, sonicate for 2 min, centrifuge at 4000 g for 5 min. Mix 7.5 mL sample extract and 40 mL 10% NaCl and add to SPE cartridge, wash with 1 mL 10 mM sulfuric acid, wash with 2 mL air, elute with 2 mL acidic MeCN. Place eluate in a washed tube and evaporate to 300 μL at 70° under a stream of nitrogen, mix gently, add 1 mL n-hexane, mix on a vibromixer for 30 s, centrifuge at 2000 g, inject a 50 μL aliquot of the aqueous phase. (Acidic MeCN was 1 mL 50 mM sulfuric acid and 100 mL MeCN. The washed tube was prepared by rinsing with concentrated ammonia, water, and acetone and drying under a stream of nitrogen.)

HPLC VARIABLES

Guard column: 10 × 2.1 37-50 μm Bondapak C18
Column: 300 × 3.9 Bondapak C18
Mobile phase: MeCN:water 55:45 containing 2.46 g/L anhydrous sodium acetate, pH adjusted to 6.5 with acetic acid
Flow rate: 1.2
Injection volume: 50
Detector: UV 240

CHROMATOGRAM

Retention time: 7.5
Limit of detection: 1 ng/g

OTHER SUBSTANCES

Extracted: azaperol, carazolol, acepromazine, xylazine, haloperidol, propiomazine, chlorpromazine

KEY WORDS

SPE; pig; kidney

REFERENCE

Keukens,H.J.; Aerts,M.M.L. Determination of residues of carazolol and a number of tranquilizers in swine kidney by high-performance liquid chromatography with ultraviolet and fluorescence detection, *J.Chromatogr.*, **1989**, *464*, 149–161.

SAMPLE

Matrix: tissue

Sample preparation: Condition a Bond-Elut C18 SPE cartridge with 5 mL MeOH and 5 mL water. Cut pig kidney or liver into small pieces and homogenize. 5 g Homogenate + 10 mL MeCN, shake, vortex for 30 s, sonicate for 3 min, vortex for 30 s, sonicate for 3 min, centrifuge at 10000 g for 20 min. Add 7.5 mL supernatant + 40 mL 10% NaCl to the SPE cartridge at about 1 mL/min, do not allow cartridge to dry out, wash with 850 μL 10 mM sulfuric acid, dry with air, elute with 3.5 mL acidic MeCN. Evaporate the

eluate to dryness under a stream of nitrogen at 50°, reconstitute the residue in 300 μL 10 mM sulfuric acid, vortex briefly, add 1 mL hexane, vortex for 30 s, centrifuge at 2000 g for 5 min, inject an aliquot of the aqueous layer. (Acidic MeCN was 1 mL 50 mM sulfuric acid in 100 mL MeCN.)

HPLC VARIABLES

Guard column: Hypersil 5 μm SAS C1
Column: 250 mm long 5 μm Hypersil SAS C1
Mobile phase: MeCN:water 50:50 containing 0.77 g/L ammonium acetate
Flow rate: 2
Detector: E, ESA Model 5100A Coulochem, first electrode +0.4 V, second electrode (which was monitored) +0.7 V, Model 5020 guard cell after pump but before injector at +0.75 V

CHROMATOGRAM

Retention time: 6.5
Limit of detection: 2 ng/g

OTHER SUBSTANCES

Extracted: azaperol, acepromazine, carazolol, xylazine, haloperidol, propriomazine, chlorpromazine

KEY WORDS

SPE; pig; kidney; liver

REFERENCE

Rose,M.D.; Shearer,G. Determination of tranquilisers and carazolol residues in animal tissue using high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.*, **1992**, *624*, 471–477.

SAMPLE

Matrix: urine
Sample preparation: Adjust 75 mL urine to pH 10 with 10 mL saturated borate buffer and concentrated ammonium hydroxide, extract with 100 mL dichloromethane:isopropanol 96:4. Extract the organic layer with 100 mL 100 mM HCl, adjust the pH of the aqueous layer to 10, extract the aqueous layer with 100 mL hexane:isopropanol 96:4. Dry the extract at 50° under a stream of nitrogen, reconstitute the residue in 500 μL MeOH, inject an aliquot.

HPLC VARIABLES

Column: 250 × 2.1 5 μm Suplex pKb-100 (Supelco)
Mobile phase: Gradient. MeCN:50 mM pH 10 ammonium acetate from 20:80 to 100:0 over 20 min.
Flow rate: 0.2
Detector: MS, Sciex API III triple quadrupole LC-MS-MS, heated nebulizer interface at 400°, corona discharge current 3 μA, orifice diameter 125 μm, collision induced dissociation using argon, argon curtain thickness was 500×10^{12} molecules/cm^2, collision energy 50 eV, positive ion mode

CHROMATOGRAM

Retention time: 20.1

OTHER SUBSTANCES

Extracted: metabolites

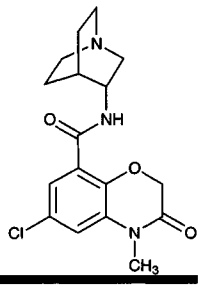
KEY WORDS

horse; LC-MS

REFERENCE

Chui,Y.C.; Esaw,B.; Laviolette,B. Investigation of the metabolism of azaperone in the horse, *J.Chromatogr.B*, **1994**, *652*, 23–33.

Azasetron



Molecular formula: $C_{17}H_{20}ClN_3O_3$
Molecular weight: 349.82
CAS Registry No.: 123040-69-7, 123040-16-4 (HCl)
Merck Index: 933

SAMPLE

Matrix: blood
Sample preparation: Add 20 μL 2 M NaOH and 2 mL dichloroethane to 200 μL serum. Shake for 10 min, centrifuge at 3000 rpm for 5 min. Remove a 1.6 mL portion of the upper organic layer, add 80 μL 100 mM HCl, and 1.5 mL hexane, centrifuge at 3000 rpm for 5 min. Remove the upper organic layer, inject a 50 μL aliquot of the lower layer.

HPLC VARIABLES

Column: 150 × 4 SenshuPak ODS-0151-N
Mobile phase: MeCN:THF:100 mM pH 5 ammonium acetate 9.8:6.2:84
Column temperature: 50
Flow rate: 1
Injection volume: 50
Detector: F ex 318 em 382

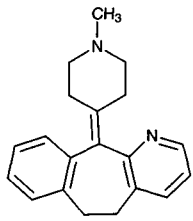
KEY WORDS

rabbit; serum; pharmacokinetics

REFERENCE

Moriyama,Y.; Arimori,K.; Nakano,M. Absorption characteristics of azasetron from rectal and oral routes in rabbits, *Biol.Pharm.Bull.*, **1997**, *20*, 701–703.

Azatadine



Molecular formula: $C_{20}H_{22}N_2$
Molecular weight: 290.41
CAS Registry No.: 3964-81-6, 3978-86-7 (maleate)
Merck Index: 934
Lednicer No.: 2 424

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 10.70 (A), 4.55 (B)

OTHER SUBSTANCES

Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphenoxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, spironolactone, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
also details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine
Sample preparation: 2 mL Urine + 200 μL 5 μg/mL 8-chloroazatadine in water + 1 mL 1 M NaOH + 6 mL diethyl ether, shake in a reciprocal shaker for 10 min, centrifuge at 1600 g for 5 min, freeze in dry ice/acetone. Remove the organic layer and add it to 500 μL 50 mM sulfuric acid, shake on a reciprocal shaker for 10 min, centrifuge at 1600 g for 5 min, freeze in dry ice/acetone, discard the organic layer. Add the aqueous layer to 1 mL 1 M NaOH, extract with 6 mL hexane, centrifuge, freeze in dry ice/acetone. Remove the organic layer and add it to 100 μL concentrated ammonium hydroxide. Evaporate to dryness under a stream of nitrogen at 45°, dissolve the residue in 500 μL mobile phase, inject a 200 μL aliquot.

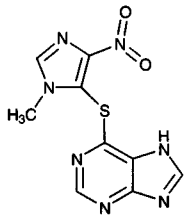
HPLC VARIABLES
Guard column: silica Guard-Pak (Waters)
Column: 300 × 3.9 10 μm μBondapak CN
Mobile phase: MeCN:50 mM pH 7.5 KH_2PO_4 90:200
Flow rate: 2
Injection volume: 200
Detector: UV 214

CHROMATOGRAM
Retention time: 7.5
Internal standard: 8-chloroazatadine (10.5)
Limit of quantitation: 10 ng/mL

OTHER SUBSTANCES
Extracted: metabolites
Simultaneous: chlorpheniramine, brompheniramine
Noninterfering: pseudoephedrine, phenylpropanolamine

REFERENCE
Alton,K.B.; Petruzzi,R.F.; Patrick,J.E. High-performance liquid chromatographic assay for azatadine in human urine, *J.Chromatogr.*, **1987**, *385*, 249–259.

Azathioprine



Molecular formula: $C_9H_7N_7O_2S$
Molecular weight: 277.27
CAS Registry No.: 446-86-6
Merck Index: 935
Lednicer No.: 2 464

SAMPLE

Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 2.5 mL MeOH and 5 mL 0.2% acetic acid. Mix 1 mL plasma with 40 μL saturated EDTA solution, add to the SPE cartridge. Wash with 2 mL 0.2% acetic acid, centrifuge at 2200 g for 5 min to remove excess water, elute with 2 mL MeOH, evaporate to dryness under a stream of nitrogen at 37°. Reconstitute the residue with 200 μL mobile phase, vortex for 30 s, centrifuge at 11000 g for 5 min, inject an 80 μL aliquot of the supernatant. (Prepare saturated EDTA solution by vortexing 2.5 g disodium (?) EDTA in 25 mL water for 5 min.)

HPLC VARIABLES

Guard column: 12.5 × 4 Zorbax ODS
Column: 200 × 4.6 5 μm Hypersil ODS
Mobile phase: Gradient. A was MeCN:1 mM triethylamine 0.8:99.2, adjusted to pH 3.2 with phosphoric acid. B was MeCN:1 mM triethylamine 20:80, adjusted to pH 3.2 with phosphoric acid. A:B 100:0 for 5 min, to 50:50 over 2.5 min, maintain at 50:50 for 11.5 min to 100:0 over 2 min, re-equilibrate at initial conditions for 9 min
Flow rate: 1.5
Injection volume: 80
Detector: UV 340

CHROMATOGRAM

Retention time: 15
Limit of quantitation: 5 ng/mL

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

SPE; plasma; pharmacokinetics

REFERENCE

Van Os,E.C.; McKinney,J.A.; Zins,B.J.; Mays,D.C.; Schriver,Z.H.; Sandborn,W.J.; Lipsky,J.J. Simultaneous determination of azathioprine and 6-mercaptopurine by high-performance liquid chromatography, *J.Chromatogr.B*, **1996**, *679*, 147–154.

SAMPLE

Matrix: blood
Sample preparation: 500 μL Serum + 25 ng 9-methylazathioprine + 4.5 mL ethyl acetate, vortex for 1 min, centrifuge for 1 min, repeat extraction. Combine the organic layers and evaporate them to dryness under reduced pressure at 35°, reconstitute the residue in 250 μL mobile phase, vortex for 10 s, sonicate for 10 min, inject a 200 μL aliquot.

HPLC VARIABLES

Guard column: 4 × 4.6 5 μm LiChrospher 100 RP 18
Column: 250 × 4.6 5 μm LiChrospher 60 Rp-select B

Mobile phase: MeCN:10 mM pH 2.3 potassium phosphate buffer 12:88 (Flush with MeCN: buffer 50:50 for 2 min after each run.)
Column temperature: 22
Flow rate: 1
Injection volume: 200
Detector: UV 285

CHROMATOGRAM

Retention time: 16
Internal standard: 9-methylazathioprine (Add 400 mg anhydrous potassium carbonate and 200 μL methyl iodide to a solution of 220 mg azathioprine in 7 mL DMF at 0-5°, stir under nitrogen at 24 h, add 14 mL water, neutralize with 1 M HCl and sodium bicarbonate solution, filter, wash the solid with water, dry under vacuum to give 9-methylazathioprine (mp 174-5°). Purify by precipitating from DMF solution with water.) (29)
Limit of quantitation: 2.5 ng/mL

OTHER SUBSTANCES

Noninterfering: 6-mercaptopurine

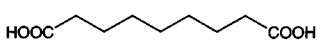
KEY WORDS

serum; pharmacokinetics

REFERENCE

Binscheck,T.; Meyer,H.; Wellhoner,H.H. High-performance liquid chromatographic assay for the measurement of azathioprine in human serum samples, *J.Chromatogr.B*, **1996**, *675*, 287–294.

Azelaic acid



Molecular formula: $C_9H_{16}O_4$
Molecular weight: 188.22
CAS Registry No.: 123-99-9
Merck Index: 938

SAMPLE

Matrix: blood
Sample preparation: Condition a 500 mg Bond Elut NH2 SPE cartridge with 3 mL MeOH and 12 mL 100 mM pH 7 NaH_2PO_4 buffer. Add 1 mL plasma to the SPE cartridge, wash with 3 mL water, wash with 3 mL MeCN, dry under vacuum, elute with 1 mL 500 mM formic acid in MeCN. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute with 100 μL 50 mM triethylamine in MeCN, vortex for 1 min, add 50 μL 60 mM ethyl chloroformate in MeCN, vortex for 1 min, add 200 μL 3 mM L-leucine-(4-methyl-7-coumarinylamide) in MeOH, vortex for 1 min, let stand for 4 min, evaporate to dryness under a stream of nitrogen, reconstitute with 200 μL MeCN:water 50:50, inject a 5-15 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Axxiom octyl (Richard Scientific, Novato)
Mobile phase: Gradient. A was 1 L water containing 2 mL 85% phosphoric acid. B was 1 L MeCN containing 2 mL 85% phosphoric acid. A:B 55:45 for 15 min, to 10:90 over 15 min.
Injection volume: 5-15
Detector: F ex 330 em 390

CHROMATOGRAM

Retention time: 30

OTHER SUBSTANCES

Extracted: dodecanedioic acid, hexadecanedioic acid, pimelic acid, tetradecanedioic acid

KEY WORDS

plasma; derivatization; SPE; dog; human

REFERENCE

Levai,F.; Liu,C.-M.; Tse,M.M.; Lin,E.T. Pre-column fluorescence derivatization using leucine-coumarnylamide for HPLC determination of mono- and dicarboxylic acids in plasma, *Acta Physiol.Hung.*, **1995**, *83*, 39–46.

SAMPLE

Matrix: blood, feces, urine
Sample preparation: Serum. 1 mL Serum + 50 μg sebacic acid, acidify to pH 1 with 1 M HCl, saturate with NaCl, extract three times with 10 mL warm (40°) ethyl acetate. Combine the extracts and dry them over anhydrous sodium sulfate, evaporate to dryness under vacuum below 40°, take up the residue in 1 mL MeCN:MeOH 80:20, add 3 mg of p-bromophenacyl bromide in MeCN, add 6 μL N,N-diisopropylethylamine, heat at 50-60° for 10-15 min, evaporate some solvent, add reaction mixture to a TLC plate (Carlo Erba Stratocrom SI-AP, 0.25 mm silica gel, activated at 120° for 20 min), develop plate in benzene:hexane 3:1 (CAUTION! Benzene is a carcinogen!), remove material remaining at origin, extract three times with 1 mL MeCN, evaporate solvent to 500 μL, inject a 20-50 μL aliquot. Urine, feces. 20-50 μL Urine or feces diluted with water + 50 μg sebacic acid, acidify to pH 1 with 1 M HCl, extract five times with 3 volumes of warm (40°) ethyl acetate. Combine the extracts and dry them over anhydrous sodium sulfate, evaporate to

dryness under vacuum below 40°, take up the residue in 1 mL MeCN:MeOH 80:20, add 3 mg of p-bromophenacyl bromide in MeCN, add 6 μL N,N-diisopropylethylamine, heat at 50-60° for 10-15 min, evaporate some solvent, add reaction mixture to a TLC plate (Carlo Erba Stratocrom SI-AP, 0.25 mm silica gel, activated at 120° for 20 min), develop plate in benzene:hexane 3:1 (CAUTION! Benzene is a carcinogen!), remove material remaining at origin, extract three times with 1 mL MeCN, evaporate solvent to 500 μL, inject a 20-50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4 5 μm RP 18 (Brownlee)
Mobile phase: Gradient. MeCN:water adjusted to pH 3.10 with phosphoric acid 60:40 for 5 min then to 100:0 over 60 min.
Column temperature: 40
Flow rate: 1
Injection volume: 20-50
Detector: UV 255

CHROMATOGRAM
Retention time: 32.2
Internal standard: sebacic acid (35.9)
Limit of detection: 0.5 ng

OTHER SUBSTANCES
Extracted: other dicarboxylic acids (C4-C13)

KEY WORDS
serum; human; rat

REFERENCE
Passi,S.; Nazzaro-Porro,M.; Picardo,M.; Mingrone,G.; Fasella,P. Metabolism of straight saturated medium chain length (C9 to C12) dicarboxylic acids, *J.Lipid Res.*, **1983**, *24*, 1140–1147.

SAMPLE
Matrix: follicles, skin
Sample preparation: Wash skin with 500 μL acetone, homogenize follicles in acetone, centrifuge at 13400 g for 5 min, remove a 400 μL portion of the supernatant, re-centrifuge. Evaporate a 30 μL aliquot, take up the residue in 1 mL MeCN:MeOH 80:20, add 3 mg of p-bromophenacyl bromide in MeCN, add 6 μL N,N-diisopropylethylamine, heat at 50-60° for 10-15 min, evaporate some solvent, add reaction mixture to a TLC plate (Carlo Erba Stratocrom SI-AP, 0.25 mm silica gel, activated at 120° for 20 min), develop plate in benzene:hexane 3:1 (CAUTION! Benzene is a carcinogen!), remove material remaining at origin, extract three times with 1 mL MeCN, evaporate solvent to 50 μL, inject a 20-50 μL aliquot.

HPLC VARIABLES
Guard column: 24 × 3.9 Bondapak C18
Column: 150 × 3.9 Novapak C18
Mobile phase: Gradient. MeCN:water adjusted to pH 3.10 with phosphoric acid 60:40 for 5 min then to 100:0 over 60 min.
Column temperature: 40
Flow rate: 1
Injection volume: 20-50
Detector: UV 254

CHROMATOGRAM
Limit of detection: 50 ng/mL

REFERENCE

Bojar,R.A.; Cutcliffe,A.G.; Graupe,K.; Cunliffe,W.J.; Holland,K.T. Follicular concentrations of azelaic acid after a single topical application, *Br.J.Dermatol.*, **1993**, *129*, 399–402.

SAMPLE

Matrix: formulations

Sample preparation: Condition a 1 g 100 μm Bakerbond Florisil SPE cartridge with 5 mL THF:hexane 40:60. Condition a 500 mg 40 μm Bakerbond C18 SPE cartridge with MeCN and MeCN:water 65:35. 200 mg Cream + 3 mL THF:hexane 40:60, sonicate, centrifuge at 3000 rpm for 2 min, repeat extraction twice. Add the supernatants to the Florisil SPE cartridge, wash with 2 mL THF:hexane 40:60, dry under vacuum, elute with two 2 mL portions of hexane:isopropanol 70:30. Evaporate the eluate to dryness under a stream of nitrogen, reconstitute the residue in 10 mL MeOH, neutralize (phenolphthalein endpoint) with 0.01% KOH in MeOH, evaporate to dryness under a stream of nitrogen at room temperature, reconstitute with 15 mL MeCN, add 5 mL 2 mM p-bromophenacyl bromide in MeCN containing 100 μM 18-crown-6, add 10 mL MeCN, stir at 80° for 30 min, cool to 4°, dilute 1:50 with MeCN:water 65:35, add a 1 mL aliquot to the C18 SPE cartridge, wash with two 3 mL aliquots of MeCN:water 75:25, elute with 10 mL MeCN. Remove a 1 mL aliquot of the eluate and add it to 100 μL 85.125 μg/mL sebacic acid, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 125 × 4 5 μm LiChrospher 100-RP-18
Mobile phase: MeCN:water 75:25
Flow rate: 1
Injection volume: 5
Detector: UV 254

CHROMATOGRAM

Retention time: 6.06
Internal standard: sebacic acid (7.59)

KEY WORDS

derivatization; cream; SPE

REFERENCE

Ferioli,V.; Rustichelli,C.; Vezzalini,F.; Gamberini,G. Determination of azelaic acid in pharmaceuticals and cosmetics by RP-HPLC after pre-column derivatization, *Farmaco*, **1994**, *49*, 421–425.

SAMPLE

Matrix: formulations

Sample preparation: Ointment, lotion. Weigh out ointment or lotion equivalent to about 15 mg azelaic acid, dissolve in 100 mL MeOH, dilute an aliquot 1:5 with water. 200 μL Sample + 150 μL 20 mM tetrahexylammonium bromide in 100 mM pH 7.0 phosphate buffer + 100 μL 4.2 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, stir for 33 min at 70°, add 150 μL 20 μg/mL IS in MeCN, sonicate for 1 min, inject a 50 μL aliquot into mobile phase A. Ointment. Dissolve in MeCN to give a concentration of 18 μg/mL. 100-200 μL Sample + 100 μL 4.2 mg/mL 2-bromoacetyl-6-methoxynaphthalene in MeCN + 100 μL 1% triethylamine in MeCN, heat at 40° for 40 min, reconstitute in 150 μL 40 μg/mL IS in Mobile Phase B and 450 μL mobile phase B, sonicate for 1 min, inject a 50 μL aliquot into mobile phase B. Powder. Weigh out powder equivalent to about 15 mg azelaic acid, dissolve in 100 mL MeOH, sonicate for 10 min, centrifuge at 4000 rpm for 20 min, filter the supernatant, dilute an aliquot of the filtrate 1:5 with water. 200 μL Sample + 150 μL 20 mM tetrahexylammonium bromide in 100 mM pH 7.0 phosphate buffer + 100 μL 4.2 mg/mL 2-bromoacetyl-6-methoxynaphthalene in acetone, stir for 33 min at 70°, add 150 μL 20 μg/mL IS in MeCN, sonicate for 1 min, inject a 50 μL aliquot into mobile phase A. (Synthesis of 2-bromoacetyl-6-methoxynaphthalene is as follows. Stir

equimolar amounts of 2-acetyl-6-methoxynaphthalene (6'-methoxy-2'-acetonaphthone, Aldrich) and methyltriphenylphosphonium tribromide in anhydrous THF under nitrogen at room temperature for 1 h, dilute the reaction mixture with ether, wash with sodium bisulfite solution, wash with water (Phosphorus and Sulfur 1985, 25, 357). [Bromination can also be achieved with phenyltrimethylammonium tribromide over 3 h but the reaction is less selective.] Purify the crude product by column chromatography on silica gel using chloroform:petroleum ether 50:50 to give 2-bromoacetyl-6-methoxynaphthalene (mp 109-112°) (Chromatographia 1992, 33, 13).)

HPLC VARIABLES

Column: 250 × 4.6 Hypersil 5 ODS
Mobile phase: MeCN:MeOH:THF:water 38.5:28:3.5:30 (A) or 37.4:27.2:3.4:32
Column temperature: 35
Flow rate: 1.2 (A), 1.6 (B)
Injection volume: 50
Detector: F ex 300 em 460

CHROMATOGRAM

Retention time: 18
Internal standard: valproic acid 6-methoxynaphthacylester (15.5) [Prepare by dissolving 2 mmoles valproic acid and 1 mmole 2-bromoacetyl-6-methoxynaphthalene in 10 mL anhydrous MeCN, add 0.5 mL triethylamine, heat at 60° for 30 min, cool, dilute with 30 mL water, extract three times with 10 mL portions of diethyl ether. Combine the extracts, wash with 5% sodium bicarbonate, wash three times with 10 mL portions of water, dry over anhydrous sodium sulfate, evaporate under reduced pressure, recrystallize from MeOH/water to give white crystals, mp 56-7° (Chromatographia 1992, 33, 13).]

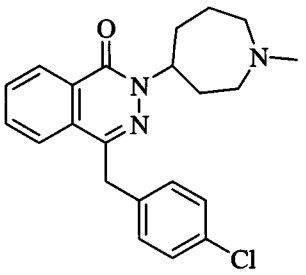
KEY WORDS

ointment; lotion; powder; derivatization

REFERENCE

Gatti,R.; Andrisano,V.; Di Pietra,A.M.; Cavrini,V. Analysis of aliphatic dicarboxylic acids in pharmaceuticals and cosmetics by liquid chromatography (HPLC) with fluorescence detection, *J.Pharm.Biomed.Anal.*, **1995**, *13*, 589–595.

Azelastine



Molecular formula: $C_{22}H_{24}ClN_3O$
Molecular weight: 381.90
CAS Registry No.: 58581-89-8, 79307-93-0 (HCl)
Merck Index: 939
Lednicer No.: 4 152

SAMPLE

Matrix: blood, tissue
Sample preparation: 1 mL Plasma or tissue homogenate (equivalent to 100 mg tissue) + 100 μL 150 ng/mL IS + 250 μL 1 M NaOH + 9 mL hexane:octanol 95:5, extract. Add the organic layer to 125 μL 0.2% acetic acid, extract, centrifuge, inject 90 μL of the aqueous layer.

HPLC VARIABLES

Column: 250 mm long 5 μm Hypersil CPS
Mobile phase: MeCN:9 mM pH 3.0 triethylammonium phosphate (sic) 50:50 (plasma) or MeCN:water:triethylamine:phosphoric acid 500:500:0.4:0.2 (tissue)
Column temperature: 60 (tissue), 40 (plasma)
Flow rate: 0.45 (plasma), 0.60 (tissue)
Injection volume: 90
Detector: F ex 215 em 360

CHROMATOGRAM

Internal standard: 4-(p-chlorobenzyl)-2-[N-methyl-2,6-ethanopiperidinyl-(4)]-1-(2H)-phthalazinone hydrochloride (9.97 (plasma), 9.61 (tissue))
Limit of quantitation: 36 ng/g (tissue), 0.288 ng/mL (plasma)

OTHER SUBSTANCES

Extracted: metabolites

KEY WORDS

plasma; guinea pig; lung

REFERENCE

Adusumalli,V.E.; Wong,K.K.; Kucharczyk,N.; Sofia,R.D. Pharmacokinetics of azelastine and its active metabolite, desmethylazelastine, in guinea pigs, *Drug Metab.Dispos.*, **1992**, *20*, 530–535.

SAMPLE

Matrix: blood, tissue
Sample preparation: Tissue. Homogenize lung tissue in ten volumes water (Tissumizer). 1 g Homogenate + 100 μL 10 μg/mL IS in 0.2% acetic acid + 100 μL 10 M NaOH + 9 mL hexane:n-octanol 95:5, rotate for 1 h at 50 rpm, centrifuge. Remove 8 mL organic layer and add it to 125 μL 0.2% acetic acid, vortex vigorously, inject an aliquot of the aqueous layer. Plasma. 1 mL Plasma + 100 μL 150 ng/mL IS in 0.2% acetic acid + 250 μL 1 M NaOH + 9 mL hexane:n-octanol 95:5, rotate for 1 h at 50 rpm, centrifuge. Remove 8 mL organic layer and add it to 125 μL 0.2% acetic acid, vortex vigorously, inject an aliquot of the aqueous layer.

HPLC VARIABLES

Column: 250 × 2 5 μm Hypersil CPS
Mobile phase: MeCN:9 mM pH 3.0 triethylammonium phosphate (sic) 50:50 (plasma) or MeCN:water:triethylamine:phosphoric acid 500:500:0.4:0.2 (tissue)

Column temperature: 60 (tissue), 40 (plasma)
Flow rate: 0.45 (plasma), 0.60 (tissue)
Detector: F ex 215 em 360

CHROMATOGRAM
Retention time: 7.88 (plasma), 8.27 (tissue)
Internal standard: 4-(p-chlorobenzyl)-2-[N-methyl-2,6-ethanopiperidinyl-(4)]-1-(2H)-phthalazinone hydrochloride (9.97 (plasma), 9.61 (tissue))
Limit of quantitation: 0.039 ng/g (tissue), 0.156 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
plasma; guinea pig; lung

REFERENCE
Langevin,C.N.; Pivonka,J.; Wichmann,J.K.; Kucharczyk,N.; Sofia,R.D. High performance liquid chromatographic determination of azelastine and desmethylazelastine in guinea pig plasma and lung tissue, *Biomed.Chromatogr.*, **1993**, *7*, 7–11.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH and dilute with water to a concentration of 20 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 conalbumin-conjugated silica gel (React 2 g Unisil Q NH_2 (Macherey-Nagel) and 3 g N,N-disuccinimidylcarbonate in 50 μL MeCN for 6 h at room temperature, filter, wash silica gel with MeCN, wash with 50 mM pH 7.5 phosphate buffer. Add silica gel to 2 g conalbumin (ovotransferrin) (from egg white) in 50 mL 50 mM pH 7.5 phosphate buffer, stir at room temperature for 6 h, filter, wash with water, wash with isopropanol: water 1:2, pack into columns.)
Mobile phase: EtOH:50 mM pH 5.0 potassium phosphate buffer 8:92
Flow rate: 1
Injection volume: 10
Detector: UV 230

CHROMATOGRAM
Retention time: 20 (d), 26 (l)

KEY WORDS
chiral

REFERENCE
Mano,N.; Oda,Y.; Miwa,T.; Asakawa,N.; Yoshida,Y.; Sato,T. Conalbumin-conjugated silica gel, a new chiral stationary phase for high-performance liquid chromatography, *J.Chromatogr.*, **1992**, *603*, 105–109.

SAMPLE
Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a solution in MeOH.

HPLC VARIABLES
Guard column: 4 × 4 5 μm LiChrospher Si-60
Column: 250 × 4 5 μm LiChrospher Si-60
Mobile phase: MeOH containing 0.033% perchloric acid

Flow rate: 0.5 for 17 min then 0.9
Injection volume: 20
Detector: F ex 210 em 360

CHROMATOGRAM

Retention time: 27
Internal standard: azelastine

OTHER SUBSTANCES

Simultaneous: flezelastine

KEY WORDS

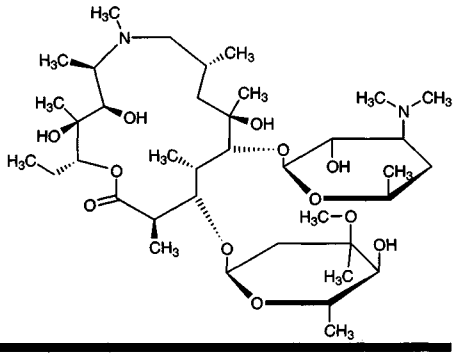
azelastine is IS

REFERENCE

Paris,S.; Blaschke,G.; Locher,M.; Borbe,H.O.; Engel,J. Investigation of the stereoselective in vitro metabolism of the chiral antiasthmatic/antiallergenic drug flezelastine by high-performance liquid chromatography and capillary zone electrophoresis, *J.Chromatogr.B*, **1997**, *691*, 463–471.

Azithromycin

Molecular formula: $C_{38}H_{72}N_2O_{12}$
Molecular weight: 749.00
CAS Registry No.: 83905-01-5
Merck Index: 946



SAMPLE

Matrix: blood
Sample preparation: Mix 200 μL plasma with 50 μL IS, add 200 μL 100 mM sodium carbonate, vortex for 30 s, add 3.5 mL MTBE, mix for 20 min, centrifuge at 2000 g for 10 min. Evaporate the MTBE layer to dryness at 37°, reconstitute the residue in 200 μL mobile phase, mix for 20 min, centrifuge, inject an 80 μL aliquot.

HPLC VARIABLES

Column: 150 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:50 mM pH 7.5 phosphate buffer 55:45
Flow rate: 1
Injection volume: 80
Detector: E, ESA Coulochem 5100 A, ESA 5010 dual electrode analytical cell at +680 mV and +780 mV, ESA 5020 guard cell +1.0 V

CHROMATOGRAM

Internal standard: clarithromycin
Limit of detection: 10 ng/mL

KEY WORDS

plasma; pharmacokinetics

REFERENCE

Patel,K.B.; Xuan,D.; Tessier,P.R.; Russomanno,J.H.; Quintiliani,R.; Nightingale,C.H. Comparison of bronchopulmonary pharmacokinetics of clarithromycin and azithromycin, *Antimicrob.Agents Chemother.*, **1996**, *40*, 2375–2379.

SAMPLE

Matrix: blood, bronchoalveolar lavage fluid
Sample preparation: Blood. Centrifuge 10 mL blood at 2500 g, separate the serum. Peripheral blood monocytes (PBM). Expose the PBM pellet to several freeze-thaw cycles, dilute the cell pellet in 1 mL saline with 1 mL serum and saline to volume 5 mL. Bronchoalveolar lavage (BAL) fluid. Expose the BAL pellet to several freeze-thaw cycles, dilute sample with water to volume 50 mL, freeze and thaw.

HPLC VARIABLES

Column: 50 × 4.6 5 μm Chromegabond Alkylphenyl aluminum oxide (E.S. Industries, N.J.)
Mobile phase: MeCN:20 mM pH 10 Na_3PO_4 75:25
Flow rate: 1.5
Detector: E, Bioanalytical Systems, dual glassy carbon electrodes 600 and 850 mV, Ag/AgCl reference electrode

CHROMATOGRAM

Limit of detection: 2 ng/mL (BAL and leucocytes), 10 ng/mL (serum)

KEY WORDS
serum; pharmacokinetics

REFERENCE
Olsen,K.M.; San Pedro,G.S.; Gann,L.P.; Gubbins,P.O.; Halinski,D.M.; Campbell,G.D.,Jr. Intrapulmonary pharmacokinetics of azithromycin in healthy volunteers given five oral doses, *Antimicrob.Agents Chemother.*, **1996**, *40*, 2582–2585.

SAMPLE
Matrix: blood, gastric juice, gastric mucosa, saliva, vitreous humor
Sample preparation: Homogenize 5-20 mg gastric mucosa in 300 μL 10 mM pH 7.4 sodium phosphate buffer with sonication. Add 500 ng roxithromycin in MeOH:water 50:50 to 500 μL plasma, serum, saliva, gastric juice, leucocytes lysate, vitreous humor, or 300 μL gastric mucosa homogenate, vortex, add 200 μL 100 mM sodium carbonate and 3 mL MTBE, shake thoroughly (5 × 2 s in an SMI Multi-tube vortexer), centrifuge at 1000 g for 5 min, freeze the aqueous layer in liquid nitrogen or in a freezer at -70° for 15 min. Evaporate the upper organic layer to dryness in a centrifugal vacuum evaporator (Jouan RC 10.22), reconstitute the residue in 250 μL MeOH:water 50:50, inject a 20-50 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Zorbax SB CN
Mobile phase: MeCN:MeOH:50 mM Na_2HPO_4 and NaH_2PO_4 buffer 52.2:4.3:43.5 (pH 6.8) (The mobile phase was a mixture of 600 mL MeCN, 50 mL MeOH and 500 mL 50 mM Na_2HPO_4 and NaH_2PO_4 buffer.)
Column temperature: 30
Flow rate: 1
Injection volume: 20-50
Detector: E, ESA Coulochem II, guard cell +1.0 V, screening cell E1 +0.50 V, analytical cell E2 +0.80 V

CHROMATOGRAM
Retention time: 12
Internal standard: roxithromycin (5.5), clarithromycin (10)
Limit of detection: 10 ng/mL

KEY WORDS
gastric juice; pharmacokinetics; plasma; saliva; serum

REFERENCE
Kees,F.; Spangler,S.; Wellenhofer,M. Determination of macrolides in biological matrices by high-performance liquid chromatography with electrochemical detection, *J.Chromatogr.A*, **1998**, *812*, 287–293.

SAMPLE
Matrix: tears
Sample preparation: Add 10 μL 20 μ g/mL IS and 500 μL of 60 mM sodium carbonate to 50 μL tears. Extract with 5 mL of MTBE. Evaporate the organic layer to dryness under a stream of nitrogen at room temperature. Reconstitute the sample in 100 μL MeCN: water 50:50, inject an 80 μL aliquot.

HPLC VARIABLES
Guard column: 4 μm Nova-Pak C18
Column: 100 × 8 4 μ m Nova-Pak C18 radial compression
Mobile phase: MeCN:MeOH:buffer 19:9:75 (Buffer was 35 mM Na_2HPO_4 containing 5 mM tetrabutylammonium phosphate and 5 mM sodium perchlorate, adjusted to pH 7.0 with phosphoric acid. Mobile phase was recycled and the buffer was prepared fresh every other day.)

Column temperature: 26
Flow rate: 4.0
Injection volume: 80
Detector: E, Coulochem 11, 5021 conditioning cell 0.7 V, 5011 analytical cell at 0.85 V

CHROMATOGRAM

Internal standard: n-propylazithromycin
Limit of quantitation: 100 ng/mL

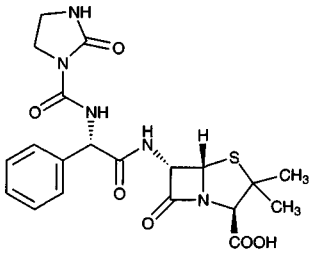
OTHER SUBSTANCES

Extracted: metabolites

REFERENCE

Raines,D.A.; Yusuf,A.; el-Yazigi,A. Simultaneous analysis of azithromycin and two of its metabolites in human tears (Abstract 2501), *Pharm.Res.*, **1997**, *14*, S377–S377.

Azlocillin



Molecular formula: $C_{20}H_{23}N_5O_6S$
Molecular weight: 461.50
CAS Registry No.: 37091-66-0, 37091-65-9 (monosodium salt)
Merck Index: 947
Lednicer No.: 3 206

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Serum. 0.5 mL serum + 0.5 mL MeCN mix in 7 mL tube on vortex mixer; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; transfer supernatant to another tube, add 7 aliquots dichloromethane; equilibrate 10 min; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; inject aliquot of upper aqueous layer. Urine. Centrifuge urine and dilute 1:20. Bile. Centrifuge bile and dilute 1:10.

HPLC VARIABLES

Column: 75 × 4.6 3 μm octadecylsilane
Mobile phase: 20:80 MeCN:20 mM ammonium acetate adjusted to pH 5 with glacial acetic acid
Flow rate: 1
Injection volume: 5
Detector: UV 214

CHROMATOGRAM

Retention time: 2.2
Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Also analyzed: ampicillin, aztreonam, cefmenoxime, cefoperazone, cefsulodin, cefotaxime, ceftazidime, ceftriaxone, cloxacillin, desacetylcefotaxime, mezlocillin, penicillin G, piperacillin, ticarcillin

KEY WORDS

serum

REFERENCE

Jehl,F.; Birckel,P.; Monteil,H. Hospital routine analysis of penicillins, third-generation cephalosporins and aztreonam by conventional and high-speed high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 109–119.

SAMPLE

Matrix: blood, bronchial secretions
Sample preparation: Add an equal volume of digestor to the bronchial secretion, shake to fluidify sample. 1 mL Serum or diluted bronchial secretion + 1 mL MeCN, vortex, centrifuge at 5000 rpm for 10 min. Remove the supernatant and add it to 4 mL dichloromethane, vortex, centrifuge. Remove the aqueous supernatant and inject an aliquot.

HPLC VARIABLES

Column: endcapped 5 μm Lichrospher RP 18
Mobile phase: MeCN:pH 7 phosphate buffer 20:80
Flow rate: 0.8
Injection volume: 4.10
Detector: UV 220

CHROMATOGRAM
Retention time: 20

KEY WORDS
serum

REFERENCE
Condomines,M.; Mallet,M.N.; Albanese,J.; Gouin,F.; De Micco,P. A rapid high-performance liquid chromatography method for determining β-lactam antibiotics in biological fluids and tissues, *Chemioterapia*, **1987**, *6*, 251–253.

SAMPLE
Matrix: blood, urine
Sample preparation: Dilute urine 1:20 with water. 200 μL Serum or diluted urine + 1 mL 20 μg/mL mezlocillin in MeCN, vortex, centrifuge, inject a 10-20 μL aliquot of the supernatant.

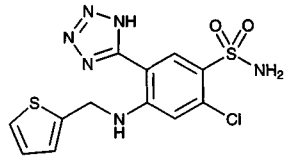
HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere C18
Mobile phase: MeOH:67 mM pH 3.0 KH_2PO_4 45:55
Flow rate: 1
Injection volume: 10-20
Detector: UV 231.1

CHROMATOGRAM
Retention time: 10.1
Internal standard: mezlocillin (12.9)

KEY WORDS
serum; pharmacokinetics

REFERENCE
Barriere,S.L.; Catlin,D.H.; Orlando,P.L.; Noe,A.; Frost,R.W. Alteration in the pharmacokinetic disposition of ciprofloxacin by simultaneous administration of azlocillin, *Antimicrob.Agents Chemother.*, **1990**, *34*, 823–826.

Azosemide



Molecular formula: $C_{12}H_{11}ClN_6O_2S_2$
Molecular weight: 370.84
CAS Registry No.: 27589-33-9
Merck Index: 953
Lednicer No.: 3 27

SAMPLE

Matrix: blood, tissue, urine
Sample preparation: Plasma, urine. 50 µL Plasma or urine + 125 µL MeCN, vortex, centrifuge at 9000 g for 10 min, inject 50 µL of the supernatant. Tissue. Homogenize with four volumes of 0.9% NaCl (Tissuemizer), centrifuge at 9000 g for 10 min. Remove 50 µL of the supernatant and add it to 125 µL MeCN, vortex, centrifuge at 9000 g for 10 min, inject a 50 µL aliquot of the supernatant.

HPLC VARIABLES

Column: 300 × 3.9 10 µm C18 (Waters)
Mobile phase: MeCN:30 mM phosphoric acid 40:50
Flow rate: 1.5
Injection volume: 50
Detector: UV 240

CHROMATOGRAM

Retention time: 6.0
Limit of detection: 50 ng/mL

OTHER SUBSTANCES

Extracted: metabolites
Noninterfering: furosemide, bumetanide, hydrochlorothiazide, amiloride, spironolactone

KEY WORDS

plasma; human; rabbit; rat; blood; liver; lung; heart; brain; kidney; muscle; stomach; intestine; spleen; pharmacokinetics

REFERENCE

Lee,S.H.; Lee,M.G. Determination of azosemide and its metabolite in plasma, blood, urine and tissue homogenates by high-performance liquid chromatography, *J.Chromatogr.B*, **1994**, *656*, 367–372.

SAMPLE

Matrix: blood, urine
Sample preparation: Urine. Filter (0.45 µm). Remove a 300 µL aliquot and add it to 300 µL water and 50 µL 412 µg/mL phenobarbital in EtOH, vortex, inject a 10 µL aliquot. Serum. 300 µL (?) Serum + 20 µL 412 µg/mL phenobarbital in EtOH + 400 µL MeCN, mix, centrifuge. Remove the supernatant and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 50-100 µL buffer, inject a 5-20 µL aliquot.

HPLC VARIABLES

Guard column: 70 × 2.1 CO:Pell ODS (Whatman)
Column: 250 × 4.6 5 µm Zorbax ODS C18
Mobile phase: Gradient. MeCN:buffer from 10:90 to 40:60 over 10 min, maintain at 40:60 for 2 min, re-equilibrate for 5 min. (Buffer was 0.6 mL glacial acetic acid in 1 L water, adjust pH to 4.05 with 4 M NaOH.)
Flow rate: 2
Injection volume: 5-20

Detector: UV 239

CHROMATOGRAM
Retention time: 10
Internal standard: phenobarbital (11)
Limit of detection: 50 ng/mL

OTHER SUBSTANCES
Noninterfering: acetaminophen, aspirin, chlorothiazide, chlorpromazine, hydrochlorothiazide, procainamide, quinidine, sulfamethoxazole, theophylline, tolbutamide

KEY WORDS
serum; pharmacokinetics

REFERENCE
Seiwell,R.; Brater,C. Separation and analysis of azosemide in urine and in serum by high-performance liquid chromatography, *J.Chromatogr.*, **1980**, *182*, 257–261.

SAMPLE
Matrix: blood, urine
Sample preparation: 100 μL Plasma or urine + 250 μL MeCN, vortex, centrifuge, inject an aliquot of the supernatant.

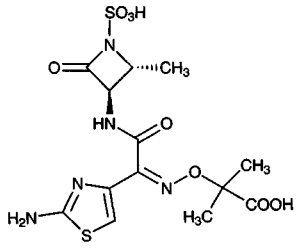
HPLC VARIABLES
Column: 300 × 3.9 10 μm C18 (Waters)
Mobile phase: MeCN:30 mM phosphoric acid 40:50
Flow rate: 1.5
Detector: UV 240

CHROMATOGRAM
Retention time: 6
Limit of detection: 90 ng/mL

KEY WORDS
rabbit; plasma; pharmacokinetics

REFERENCE
Lee,S.H.; Shin,W.G.; Lee,M.G.; Kim,N.D. Arterial and venous blood sampling in pharmacokinetic studies: azosemide in rabbits, *Biopharm.Drug Dispos.*, **1994**, *15*, 305–316.

Aztreonam



Molecular formula: $C_{13}H_{17}N_5O_8S_2$
Molecular weight: 435.44
CAS Registry No.: 78110-38-0
Merck Index: 955
Lednicer No.: 4 193, 195

SAMPLE

Matrix: bile, blood, urine
Sample preparation: Serum. 0.5 mL serum + 0.5 mL MeCN mix in 7 mL tube on vortex mixer; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; transfer supernatant to another tube, add 7 aliquots dichloromethane; equilibrate 10 min; shake by rotation (20 rpm) 10 min; centrifuge 10 min 1000 g; inject aliquot of upper aqueous layer. Urine. Centrifuge urine and dilute 1:20. Bile. Centrifuge bile and dilute 1:10.

HPLC VARIABLES

Column: 150 × 4.6 5 μm Ultrasphere ODS
Mobile phase: 33:67 MeCN:10 mM ammonium acetate + 5 mM tetrabutylammonium bromide adjusted to pH 7 with glacial acetic acid
Flow rate: 1
Injection volume: 20
Detector: UV 254

CHROMATOGRAM

Retention time: 4.2
Limit of detection: 500 ng/mL

OTHER SUBSTANCES

Also analyzed: ampicillin, azlocillin, cefmenoxime, cefoperazone, cefsulodin, cefotaxime, ceftazidime, ceftriaxone, cloxacillin, desacetylcefotaxime, mezlocillin, penicillin G, piperacillin, ticarcillin

KEY WORDS

serum

REFERENCE

Jehl,F.; Birckel,P.; Monteil,H. Hospital routine analysis of penicillins, third-generation cephalosporins and aztreonam by conventional and high-speed high-performance liquid chromatography, *J.Chromatogr.*, **1987**, *413*, 109–119.

SAMPLE

Matrix: blood, urine
Sample preparation: Serum. Dilute serum with an equal volume of MeCN, centrifuge at 15000 g for 2 min, inject a 50-200 μL aliquot of the supernatant. Urine. Dilute urine tenfold with 5 mM pH 3.0 tetrabutylammonium hydrogen sulfate, inject a 50-200 μL aliquot.

HPLC VARIABLES

Guard column: 30 × 3.9 Bondapak C18/Corasil
Column: 300 × 3.9 μBondapak C18
Mobile phase: Human serum, human urine. MeCN:5 mM tetrabutylammonium hydrogen sulfate and 5 mM ammonium sulfate adjusted to pH 3.0 with 1 M K_2HPO_4 20:80; Mouse serum, mouse urine, monkey urine. MeCN:buffer 20:80; Rat serum, monkey serum. MeCN:buffer 35:65; Rat urine, rabbit urine. MeCN:buffer 17.5:82.5; Rabbit serum. MeCN:

buffer 25:75 (Buffer was 5 mM tetrabutylammonium hydrogen sulfate adjusted to pH 3.0 with 1 M K_2HPO_4.)
Flow rate: 2
Injection volume: 50-200
Detector: UV 293

CHROMATOGRAM
Retention time: 5 (human serum and urine)
Limit of detection: 5000 ng/mL (urine), 1000 ng/mL (serum)

KEY WORDS
serum; human; monkey; rat; mouse; rabbit

REFERENCE
Pilkiewicz,F.G.; Remsburg,B.J.; Fisher,S.M.; Sykes,R.B. High-pressure liquid chromatographic analysis of aztreonam in sera and urine, *Antimicrob.Agents Chemother.*, **1983**, *23*, 852–856.

SAMPLE
Matrix: formulations
Sample preparation: 100 μL Solution + 4.9 mL MeOH:water 20:80, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm Adsorbosphere C18
Column: 250 × 4.6 5 μm Adsorbosphere C18
Mobile phase: MeCN:5 mM pH 2.6 ammonium phosphate containing 2 mM tetrabutylammonium hydroxide 26:74
Flow rate: 1
Injection volume: 50
Detector: UV 238

CHROMATOGRAM
Retention time: 14.0

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Inagaki,K.; Gill,M.A.; Okamoto,M.P.; Takagi,J. Stability of ranitidine hydrochloride with aztreonam, ceftazidime, or piperacillin sodium during simulated Y-site administration, *Am.J.Hosp.Pharm.*, **1992**, *49*, 2769–2772.

SAMPLE
Matrix: formulations
Sample preparation: Dilute 1: 8 with water, combine a 100 μL aliquot of the diluted solution with 100 μL cimetidine solution and 200 μL water, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 3.9 × 300 μBondapak C18
Mobile phase: 5 mM tetrabutylammonium hydrogen sulfate, 7% MeCN, 14% MeOH in 10 mM phosphate buffer (pH 2.6-2.7)
Flow rate: 1
Injection volume: 20
Detector: UV 225

CHROMATOGRAM
Retention time: 7.05
Internal standard: cimetidine (3.27)

OTHER SUBSTANCES
Simultaneous: with ampicillin, sulbactam

KEY WORDS
injections; stability-indicating; saline

REFERENCE
Belliveau,P.P.; Nightingale,C.H.; Quintiliani,R. Stability of aztreonam and ampicillin sodium-sulbactam sodium in 0.9% sodium chloride injection, *Am.J.Hosp.Pharm.*, **1994**, *51*, 901–904.

SAMPLE
Matrix: formulations
Sample preparation: Add cefazolin sodium (20 mg/mL), inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:buffer 20:80 (Buffer was 5 mM tetrabutylammonium hydrogen sulfate, adjusted to pH 3 with 1 M KH_2PO_4.)
Flow rate: 2
Injection volume: 10
Detector: UV 293

CHROMATOGRAM
Retention time: 7.5
Internal standard: cefazolin (13)

KEY WORDS
injections; stability-indicating; 5% dextrose

REFERENCE
Bosso,J.A.; Prince,R.A.; Fox,J.L. Compatibility of ondansetron hydrochloride with fluconazole, ceftazidime, aztreonam, and cefazolin sodium under simulated Y-site conditions, *Am.J.Hosp.Pharm.*, **1994**, *51*, 389–391.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject a 15 μL aliquot.

HPLC VARIABLES
Guard column: 5 μm C8 (Vydac)
Column: 250 × 4.6 5 μm C8 (Vydac)
Mobile phase: MeOH:buffer 15:85 (Buffer was 50 mM KH_2PO_4 adjusted to pH 3.0 with phosphoric acid.)
Flow rate: 1
Injection volume: 15
Detector: UV 270

CHROMATOGRAM
Retention time: 7.8

OTHER SUBSTANCES
Simultaneous: vancomycin

KEY WORDS
stability-indicating; injections; 5% dextrose; saline

REFERENCE
Trissel,L.A.; Xu,Q.A.; Martinez,J.F. Compatibility and stability of aztreonam and vancomycin hydrochloride, *Am.J.Health-Syst.Pharm.*, **1995**, *52*, 2560–2564.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 6μm Zorbax CN
Mobile phase: 1.8 mM pH 4.1 copper sulphate
Flow rate: 0.7
Injection volume: 25
Detector: UV 232

CHROMATOGRAM
Retention time: 10.3
Limit of quantitation: 50 ng/mL

OTHER SUBSTANCES
Simultaneous: arginine

REFERENCE
Khedr,A. High-performance liquid chromatography of α-amino acids and aztreonam on reversed phase columns with aqueous Cu2+ as eluent, *Biomed.Chromatogr.*, **1996**, *10*, 167–171.

SAMPLE
Matrix: solutions
Sample preparation: Add 20 μL solution to 2 mL 1 mg/mL cefoperazone in water, vortex for 15 s, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 10 μm Alltech C8
Mobile phase: MeCN:buffer 23:77 containing 1.7 g/L tetrabutylammonium hydrogen sulfate, pH adjusted to 3.5 with 5 M NaOH (The buffer was 60 mL 100 mM sodium acetate and 710 mL 100 mM acetic acid.)
Flow rate: 2
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 8.0
Internal standard: cefoperazone (13.5)

OTHER SUBSTANCES
Simultaneous: degradation products

KEY WORDS
5% dextrose; saline

REFERENCE
Marble,D.A.; Bosso,J.A.; Townsend,R.J. Compatibility of clindamycin phosphate with aztreonam in polypropylene syringes and with cefoperazone sodium, cefonicid sodium, and cefuroxime sodium in partial-fill glass bottles, *Drug Intell.Clin.Pharm.*, **1988**, *22*, 54–57.